Journal of Physiology (1995), **486.1**, *pp.*223–228

The electrical activity of mouse pancreatic β-cells recorded *in vivo* shows glucose-dependent oscillations

J. V. Sánchez-Andrés, A. Gomis and M. Valdeolmillos*

Departamento de Fisiología and Instituto de Neurociencias, Facultad de Medicina, Universidad de Alicante, 03080 Alicante, Spain

1. The characteristics of the electrical activity of β-cells from islets of Langerhans recorded *in vivo* are described. For blood glucose concentrations from 4 to 11 mM, the electrical activity of pancreatic β-cells is oscillatory, with alternating depolarized and hyperpolarized phases. During the depolarized phases, action potentials are triggered.

2. The main effect of increasing glucose concentration consists of an increase in the duration of the depolarized phase. The relationship between blood glucose concentration and the percentage of time in the depolarized phase can be described by a sigmoidal function with half-activation at 6·8 mM glucose. The equivalent value obtained in parallel experiments *in vitro* is 13·3 mM, a significant rightward shift in the activation curve that suggests a role for other neural or humoral factors in determining the islet sensitivity to glucose.

3. The injection of glucose into the bloodstream produces a transitory phase of continuous electrical activity that is recorded within seconds after the change and that leads to a decrease of the glycaemia to the prestimulatory value.

4. The results demonstrate that under physiological conditions the electrical response of β-cells to glucose consists of membrane potential oscillations, validating previous data obtained with isolated preparations. Furthermore, the electrical response occurs at lower levels of glycaemia than those predicted from recordings in isolated preparations and is maximal within the physiological range of blood glucose.

The islets of Langerhans play a key role in the control of blood glucose concentration through the secretion of insulin and other hormones. Previous work *in vitro* has permitted the establishment of the basic mechanisms involved in stimulus–secretion coupling in pancreatic β-cells. At sub-stimulatory glucose concentrations, the membrane potential of the β-cells stays hyperpolarized, close to the K^+ equilibrium potential, due to the activity of ATP-regulated K^+ channels (K_{ATP}). Glucose-induced insulin secretion is initiated by the metabolism of the sugar, which leads to a rise in the ATP levels and the closure of K_{ATP} channels (Ashcroft, Harrison & Ashcroft, 1984; Cook & Hales, 1984). This produces a membrane potential depolarization and the activation of voltage-dependent calcium currents. The ensuing rise in intracellular calcium (Grapengiesser, Gylfe & Hellman, 1989; Valdeolmillos, Nadal, Contreras & Soria, 1992), by mechanisms not well understood, triggers the release of insulin. Therefore there is a direct link between changes in the membrane potential and insulin release.

The electrical activity of isolated islets of Langerhans is oscillatory (Dean & Matthews, 1968) with alternating depolarized and hyperpolarized phases (see Ashcroft & Rorsman, 1989, for a review). Furthermore, there is a positive correlation between the relative length of the depolarized phase and the amount of insulin secreted (Henquin & Meissner, 1984; Atwater, Carroll & Xu Li, 1989). Despite the fact that the properties of the electrical activity of islets of Langerhans recorded *in vitro* are relatively well characterized, such properties *in vivo* were not until now. Due to the fact that the studies in isolated islets have been performed in the absence of an intra-islet capillary circulation, the possibility that such characteristics are not representative for the *in vivo* situation has been recently suggested (Gylfe, Grapengiesser & Hellman, 1991).

Here we describe the main characteristics of the electrical activity of islets of Langerhans recorded *in vivo*, their glucose dependence and the response to sudden changes in blood glucose concentration. A preliminary account of some

* To whom correspondence should be addressed.

This manuscript was accepted as a Short Paper for rapid publication.

of these results has been reported (Sánchez-Andrés, Gomis & Valdeolmillos, 1994).

METHODS

Albino mice (8–10 weeks old, 25–35 g in weight), with free access to food and water, were anaesthetized by intraperitoneal injection of 90 mg kg^{-1} Nembutal. The degree of anaesthesia was periodically checked during the experiment by exploration of cutaneous reflexes. The experiments were carried out according to institutional guidelines. The peritoneal cavity was opened and the duodenal part of the pancreas dissected free from adherences. The vena cava and the abdominal aorta were cannulated in their most caudal parts for solution infusion and blood sample collection, respectively. During the experiment the animal was laid on its back on a heated bed maintained at 37 °C. For the electrical recording, the duodenal part of the pancreas was spread out and held slightly upwards on top of a rectangular base (6 × 20 mm) covered by a 3 mm layer of Sylgard (Dow Corning). The base was attached to a micromanipulator. The pancreas was fixed to the base by means of dissection pins, taking care not to affect the blood supply. In this way, the islets were effectively isolated from respiratory and peristaltic movements. Recordings were made from forty-eight cells, from forty-four different islets. The cells were impaled with high-resistance glass microelectrodes (80–120 MΩ) filled with a solution of 3 M potassium citrate plus 100 mM potassium chloride, and the electrical activity recorded with an Axoclamp-2A (Axon Instruments). Unfiltered records were acquired at a frequency of 300 Hz and stored on a microcomputer using Axotape (Axon Instruments) for later analysis. Samples of blood (25–50 μl) collected from the abdominal aorta were analysed for glucose concentration by the glucose oxidase method using a Beckman glucose-analyser-2.

In parallel experiments, we recorded from twenty-eight cells from isolated islets, as previously described (Sánchez-Andrés, Ripoll & Soria, 1988). Once dissected out, the islets were fixed to the base of a Perspex chamber and superfused with a modified Krebs solution containing (mM): 120 NaCl, 5 KCl, 25 $NaHCO_3$, 2·5 $CaCl_2$ and 1·1 $MgCl_2$, constantly gassed with a mixture of 95% O_2, 5% CO_2 (pH = 7·4). These experiments were done at 37 °C with different glucose concentrations ranging from 0 to 40 mM. All the reagents were of analytical grade from Sigma and Merck.

The plot presented in Fig. 3 (curves labelled *in vivo* and *in vitro*) shows the fitting of the data to a sigmoidal function with the following equation:

$$y = 100/1 + [(10^c)^d/(10^x)^d],$$

where $10^c = EC_{50}$ and d is the slope.

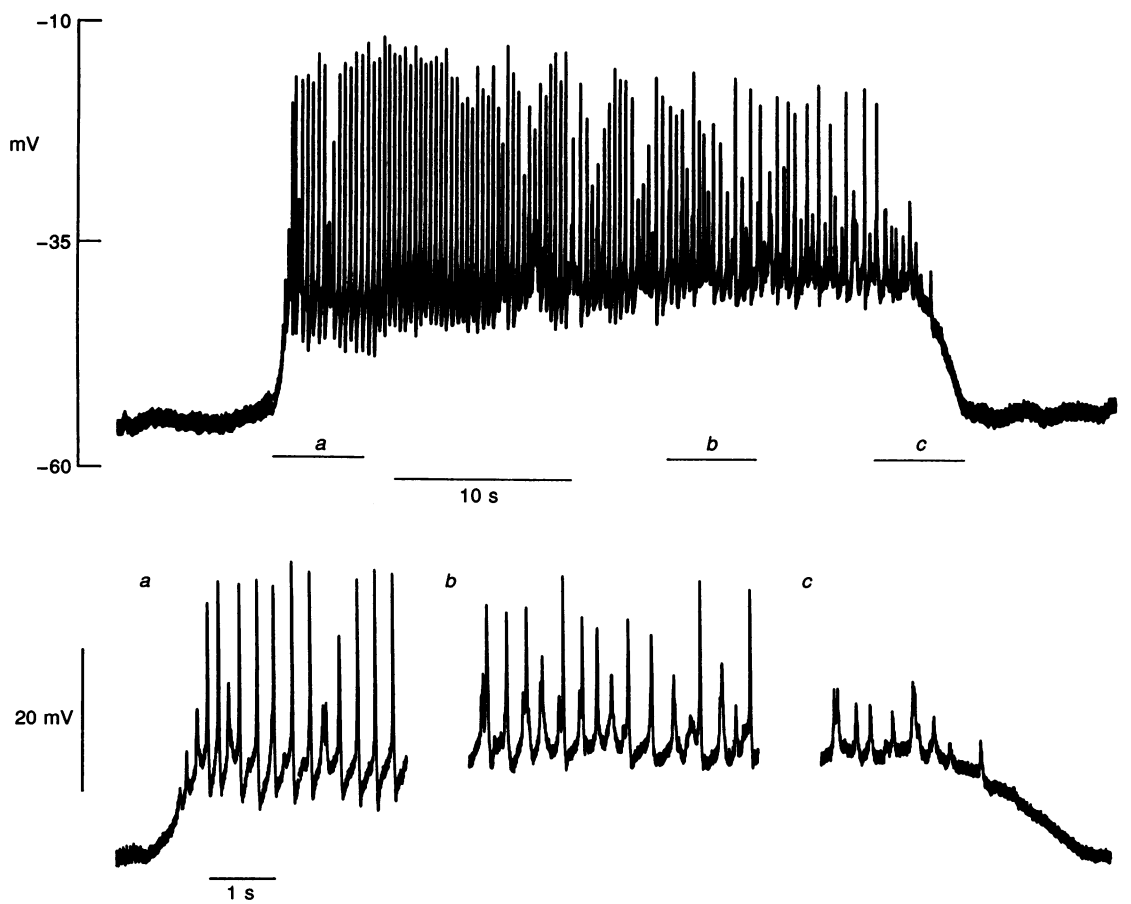

Figure 1. Electrical activity of islets of Langerhans

Upper panel, representative trace of the electrical activity of islets of Langerhans recorded *in vivo*. The lower panel shows the expanded areas indicated in the upper panel.

RESULTS

Figure 1 (upper panel) shows a representative record of the electrical activity in islets recorded intracellularly *in vivo*, consisting of membrane potential oscillations. The oscillations are composed of hyperpolarized phases (silent phases), with a membrane potential in the range of −65 to −55 mV, followed by depolarized plateau phases 10–20 mV more positive than the hyperpolarized phase. During the plateau, bursts of action potentials are generated. The amplitude of the action potentials was variable in different cells, ranging from 10 to 40 mV. The frequency of the action potentials at the beginning of the burst was 4–7 spikes s^{-1} and gradually decreased until the cell finally repolarized. This can be more clearly appreciated in the lower panel of Fig. 1, which shows expanded records of different sections of the active phase. The amplitude of the action potentials was variable throughout the burst, the largest being observed at the beginning. Also, the duration of the action potentials changed along the burst, being shorter at the beginning and lengthening as the burst

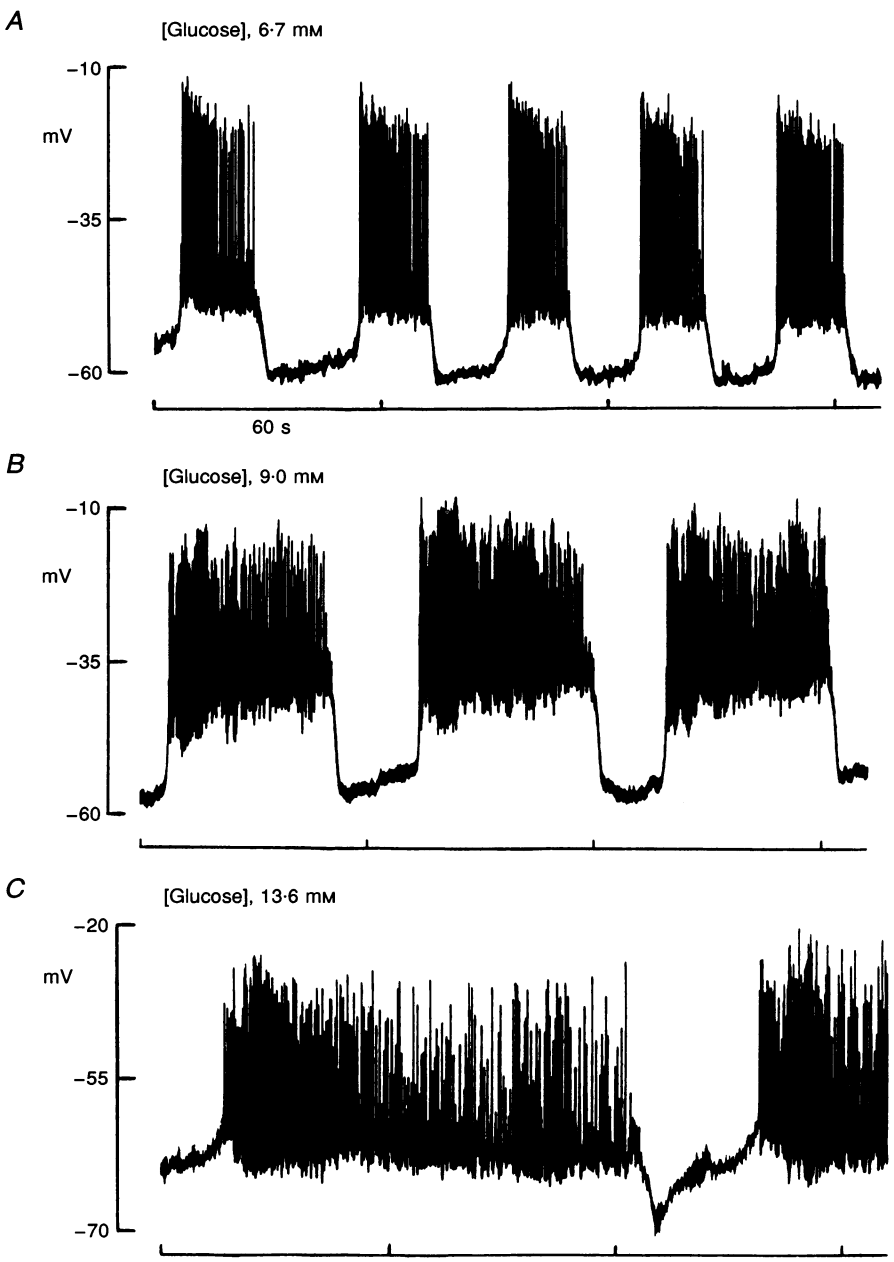

Figure 2. Patterns of electrical activity for different glycaemia levels

Specimen intracellular records of the electrical activity recorded *in vivo* from islets of Langerhans of different animals. The blood glucose concentration measured during the electrical recording is indicated in each trace.

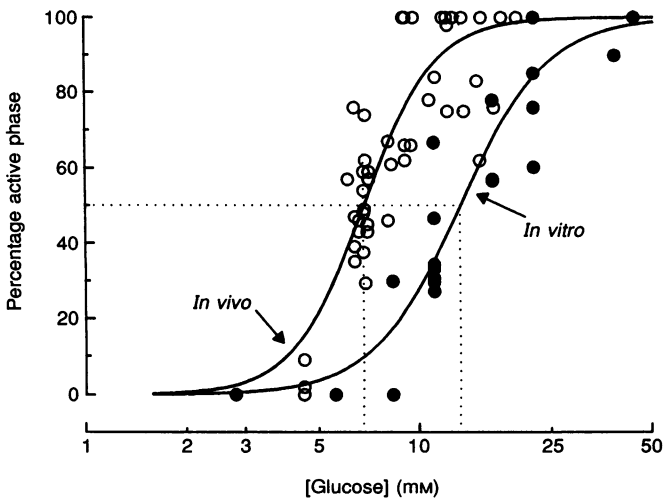

Figure 3. Comparison between the *in vivo* and *in vitro* electrical activity at different glucose concentrations

Semilogarithmic plot of the relationship between blood glucose levels and the percentage of time spent at the active phase *in vivo* (○) and *in vitro* (●). The dotted lines correspond to the EC_{50} values.

progresses. Overall, the characteristics of the oscillations recorded *in vivo* are very similar to those recorded in isolated islets of Langerhans.

Figure 2 shows electrical recordings from animals with different blood glucose concentrations measured simultaneously to the electrical recording, and indicated at the top of each record. As can be appreciated, the main effect of increased glucose levels consists of an increase in the relative length of the active phase.

Work on isolated islets has shown a positive correlation between the time spent at the depolarized plateau and insulin secretion, obtained at different glucose concentrations. Figure 3 shows a plot of the relationship between the percentage of time spent at the plateau phase (active phase) and the blood glucose concentration, obtained in the islets recorded *in vivo* (○, $n = 48$). The data were fitted to a sigmoidal curve with an $EC_{50} = 6{\cdot}80$ mM (95% confidence interval, 6·45–7·07 mM). The filled circles show data obtained from microdissected islets and recorded

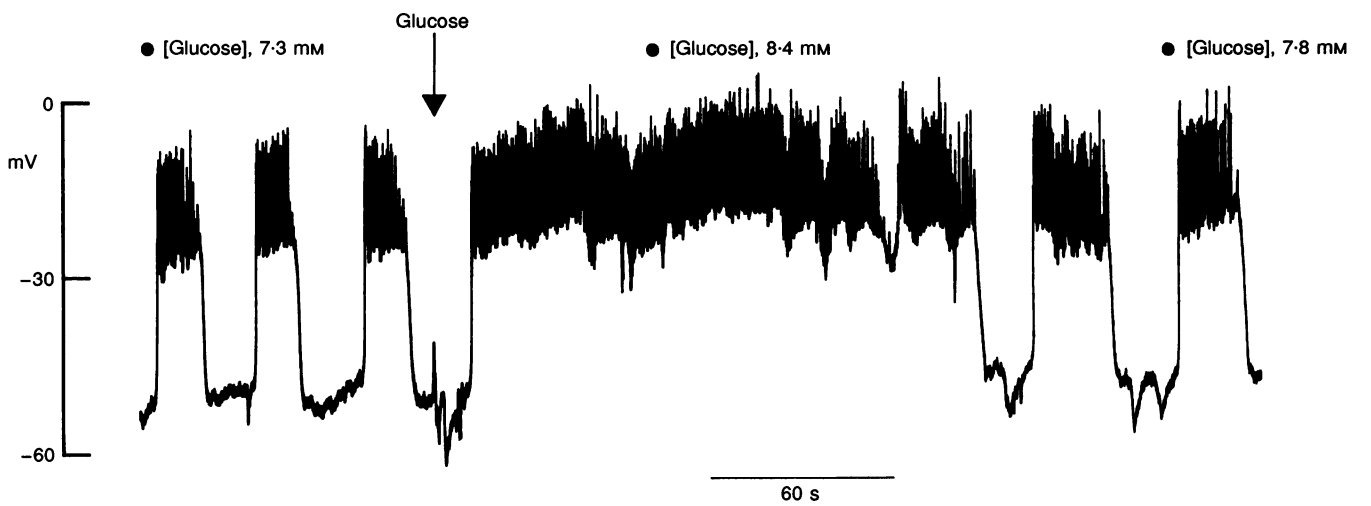

Figure 4. Effect of increasing blood glucose concentration on β-cell electrical activity

At the time indicated by the arrow, 15 μl of saline solution containing glucose (250 mM) was infused as a single bolus into the vena cava. The glucose concentration given above the record was measured from blood samples collected from the abdominal aorta at the times indicated by the dots. Representative of 10 experiments in 10 different animals.

in vitro ($n = 28$). The fitting provided an $EC_{50} = 13{\cdot}34$ (95% confidence interval, 12·02–14·45 mM), a value in agreement with recordings from other laboratories (Atwater *et al.* 1989). The differences in the EC_{50} values should be at the expense of the displacement of the *in vitro* curve to the right, as the comparison of the slopes did not show any significant difference (*in vivo* slope = 4·34; 95% confidence interval, 2·85–5·29 mM; *in vitro* slope = 3·28; 95% confidence interval, 2·28–4·28 mM). In fact, the comparison of the multiple non-linear regression equations gave $F_{(2,69)} = 63{\cdot}445$ ($P < 0{\cdot}0001$), demonstrating a significant shift.

The regulatory function of the islets of Langerhans requires rapid responses to variations in glucose. Figure 4 shows a continuous record of the electrical activity in response to changes in blood glucose concentration. In this experiment the glycaemia at the beginning of the record was 7·3 mM, associated with oscillations in the electrical activity. At the time indicated by the arrow, a bolus of glucose was injected into the cava vein, leading to an increase in blood glucose concentration and the appearance of continuous electrical activity. After a period of about 4 min, the membrane potential recovered towards its initial oscillatory pattern in parallel with the decrease in glucose concentration. The decrease in blood glucose after the glucose bolus is probably due to the increased insulin secretion as a result of the transient increase in the electrical activity.

DISCUSSION

Our results demonstrate that under physiological conditions, the islets of Langerhans show glucose-dependent oscillatory electrical activity. The oscillations recorded *in vivo* are similar to those recorded in isolated islets of Langerhans, where the action potentials have been shown to be calcium dependent (Dean & Mathews, 1970). The variability in the amplitude of the spikes through the burst is also reminiscent of *in vitro* recordings as well as the occurrence of overlapping action potentials. This may be due to the fact that β-cells are electrically coupled by gap junctions (Eddlestone, Goncalves, Bangham & Rojas, 1984; Meda, Santos & Atwater, 1986), and some of the spikes are action potentials propagated from neighbouring cells.

The half-activation value obtained *in vivo* (6·8 mM) corresponds to the normal range of glycaemia in living mice (5·0 and 7·0 mM, normal fasting and postprandial levels, respectively). This value is centred in the region of higher slope of the dose–response curve, permitting the fastest response to either an increase or a decrease in the blood glucose concentration. The difference between glucose sensitivity *in vivo* and *in vitro* stresses the importance of humoral or nervous influences in determining the islet sensitivity to glucose.

The electrical response to a step increase in the level of blood glucose is established in a few seconds, a relatively rapid response that can be expected to follow closely the postprandial glycaemia variations.

The response of the islets showed a good fitting to a single function, strongly suggesting that there are no significant differences in the sensitivity of individual islets. With this premise, we hypothesize that all the islets within the pancreas respond as a whole to changes in blood glucose concentration, and the behaviour of the system is not dependent on the recruitment of islets with different glucose sensitivities.

In vivo recording can be relevant to the understanding of the relationship between the electrical oscillations and the pulsatile insulin secretion observed in plasma (Lang, Matthews, Peto & Turner, 1979), the abnormality of which is thought to be an early phenomenon in the development of non-insulin-dependent diabetes (O'Rahilly, Turner & Matthews, 1988). Furthermore, it will allow the study of the nervous regulation of the islet function and may be used as a direct method to test the effectiveness of anti-diabetic drugs.

Ashcroft, F. M., Harrison, D. E. & Ashcroft, S. J. H. (1984). Glucose induces closure of single potassium channels in isolated rat pancreatic β-cells. *Nature* **312**, 446–448.

Ashcroft, F. M. & Rorsman, P. (1989). Electrophysiology of the pancreatic β-cell. *Progress in Biophysics and Molecular Biology* **54**, 87–143.

Atwater, I., Carroll, P. & Xu Li, M. (1989). Electrophysiology of the pancreatic β-cell. In *Molecular and Cellular Biology of Diabetes Mellitus*, vol. 1, ed. Draznin, B., Melmed, S. & LeRoith, D., pp. 49–68. Alan R. Liss, Inc., New York.

Cook, D. L. & Hales, C. N. (1984). Intracellular ATP directly blocks K^+ channels in pancreatic β-cells. *Nature* **311**, 271–273.

Dean, P. M. & Matthews, E. K. (1968). Electrical activity in pancreatic islet cells. *Nature* **219**, 389–390.

Dean, P. M. & Matthews, E. K. (1970). Electrical activity in pancreatic islet cells: effects of ions. *Journal of Physiology* **210**, 265–275.

Eddlestone, G. T., Goncalves, A., Bangham, J. A. & Rojas, E. (1984). Electrical coupling between cells in islets of Langerhans from mouse. *Journal of Membrane Biology* **77**, 1–14.

Grapengiesser, E., Gylfe, E. & Hellman, B. (1989). Three types of cytoplasmic Ca^{2+} oscillations in stimulated pancreatic β-cells. *Archives of Biochemistry and Biophysics* **268**, 404–407.

Gylfe, E., Grapengiesser, E. & Hellman, B. (1991). Propagation of cytoplasmic Ca^{2+} oscillations in clusters of pancreatic β-cells exposed to glucose. *Cell Calcium* **12**, 229–240.

Henquin, J. C. & Meissner, H. P. (1984). Significance of ionic fluxes and changes in membrane potential for stimulus–secretion coupling in pancreatic β-cells. *Experientia* **40**, 1043–1052.

Lang, D. A., Matthews, D. R., Peto, J. & Turner, R. C. (1979). Cyclic oscillations of basal plasma glucose and insulin concentration in human beings. *New England Journal of Medicine* **301**, 1023–1027.

MEDA, P., SANTOS, R. M. & ATWATER, I. (1986). Direct identification of electrophysiologically monitored cells within intact mouse islets of Langerhans. *Diabetes* **35**, 232–236.

O'RAHILLY, S., TURNER, R. C. & MATTHEWS, D. R. (1988). Impaired pulsatile secretion of insulin in relatives of patients with non-insulin-dependent diabetes. *New England Journal of Medicine* **318**, 1225–1230.

SÁNCHEZ-ANDRÉS, J. V., RIPOLL, C. & SORIA, B. (1988). Evidence that muscarinic potentiation of insulin release is initiated by an early calcium entry. *FEBS Letters* **231**, 143–147.

SÁNCHEZ-ANDRÉS, J. V., GOMIS, A. & VALDEOLMILLOS, M. (1994). *In vivo* recording of the electrical activity of islets of Langerhans. *Proceedings of the European Association for the Study of Diabetes – islet study group.* University of Tübingen, Germany.

VALDEOLMILLOS, M., NADAL, A., CONTRERAS, D. & SORIA, B. (1992). The relationship between glucose-induced K^+_{ATP} channel closure and the rise in $[Ca^{2+}]_i$ in single mouse pancreatic β-cells. *Journal of Physiology* **455**, 173–186.

Acknowledgements

We thank R. Gallego, E. Geijo and B. Soria for helpful comments on the manuscript, E. Ferrandis for help with statistics and A. Perez-Vergara and S. Moya for technical assistance. This work was supported by grants from DGICYT PM92-0115 (Dirección General de Investigación Científica y Técnica) to J.V.S.-A. and FIS 94/1345 (Fondo de Investigaciones Sanitarias de la Seguridad Social, Spain) to M.V.

Received 22 February 1995; accepted 28 April 1995.